Radicals scavenging potentials and mosquito vectors' bio-control measures from bioactive principles of the marine red algae Porteria hornemannii

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Abstract

This study investigated the presence of phytochemicals, yield of extract, total phenol and flavonoids content of the red seaweed *Porteria hornemannii*. For the extraction of metabolites from *P. hornemannii*, two different solvents like methanol and chloroform were used. In the phytochemical test, the methanol extract of *P. hornemannii* (MEPH) showed superior results in comparison with chloroform. After the crude methanol extract is purified by 86%, it is subjected to a quantitative method which, respectively, determined the total phenol content to be (1.60 ± 0.13 mg.GAE/g) and flavonoid content to be (1.302 ± 0.4 mg.GAE/g). The GC-MS analyses of MEPH revealed 20 compounds, in which, n-hexadeconic acid (Rt-27.385; peak area – 41.88%) and 9, 12, Octadecadienoic acid (Z, Z) (Rt-29.879; peak area – 19.49%) were the major ones. Based on the concentration of MEPH, both the ABTS and the hydroxyl radical scavenging assays showed increased activity. The larvicidal activity of MEPH tested against three mosquito larvae and found that only *Culex quinquefasciatus* larvae was highly sensitive (100% mortality) with the LC50 and LC90 value of 2.476 ppm and 13.207 ppm, respectively. Finally, the seaweed *P. hornemannii* could be considered for the future nutraceutical and bio-control agents, which may also be impervious to cancer and vector-borne diseases in human.

1. Introduction

Seaweed is a type of marine macroalgae that can be harvested annually as a renewable living resource. In addition to being used for food, feed, and fertilizer, seaweeds are used in many other fields. There are more than sixty trace elements found in seaweeds, which are found in higher concentrations than in terrestrial plants. Aside from bromine, vitamins, protein, and iodine, seaweeds are also rich in substances of a stimulatory, antibiotic, and antimicrobial nature. Many secondary metabolites were derived from macroalgae, including fucoxanthin, terpenes, polyphenols, steroids, halogenated ketone, alkanes, and polyphloroglucinol or bromophenols (El-Din and El-Ahwany 2016). The importance of seaweeds in the food and pharmaceutical industries is a result of their ecological and nutritional importance to both the food and pharmaceutical industries. According to several scientific studies, macroalgae have a wide range of bioactive compounds that exhibit numerous biological properties viz., anti-aging, antimicrobial antimalarial, dietary, anti-inflammatory, anticoagulant, antiallergic, antiproliferation, antibiotic, anticancer, antioxidant and hypolipidemia properties (Kumar et al. 2020) (Agregán et al. 2017) (Chan et al. 2015). Phlorotannins, a class of polyphenolic compounds found in seaweeds, are polymeric forms of phloroglucinol and have been found to possess strong antioxidant properties and possess a greater ability to scavenge free radicals than monophenols and other polyphenols commonly found in terrestrial plants (Shunmugiah et al. 2022).

There is a wide variation of factors affecting the composition of nanoalgae functional foods, such as food compatibility, nature of the solvent, time required, yield, purity, and cost, etc., which may influence the composition of macroalgae functional foods (Archambault et al. 2014). Approximately 2.5 billion people worldwide are affected by dengue disease, according to the World Health Organization (WHO), which has been reported in several tropical or subtropical countries (WHO 2012) (WHO 2017). According

to Balasubramanian et al. (2015), it is estimated that approximately two million people are affected by malaria and dengue every year in India. Commercial insecticides are commonly found on the market today. A large majority of them were developed in the 1970s and 1980s and are synthetic-like chemicals, such as organophosphates, organochlorines, and carbonates, that, when applied, could cause significant environmental issues (Ignacimuthu and David 2009). Because of their insecticidal properties, seaweeds not only inhibit/block mosquito larvae's metabolic activity, growth, and feeding behavior but also make their presence in nature more effective in blocking mosquito larvae from breeding and spreading. This is an excellent illustration of the insecticidal properties of seaweeds (Elbanna and Hegazi 2011). In other words, marine-derived natural products, such as halogenated terpenes, can be used on a large scale for the formulation of new larvicide compounds and for the development of prototype insecticides that are based upon them. There have been several studies of other compounds that are synergistic or combined with synthetic insecticides to create significant efficacy, including dichlorodiphenyltrichloroethane (DDT), 2-benzenehexachloride (BHC), and malathion (Carroll et al. 2022). Hence, the current research pertained to the antioxidant and larvicidal properties of marine macroalgae, *P. hornemannii*, through the detection of secondary metabolites, and compared its performance between different samples.

2. Materials And Methods

2.1. Sample collection

During December 2017 and January 2018, sampling of fresh and solid samples of *Porteria hornemannii*, a marine alga, was performed along the shoreline of Kilakarai, Ramnad, Tamil Nadu, India (9°14'58" N/78°47'18" E) between the low tide period and at a depth between 1 and 5 meters. It was sent to the laboratory in the form of a polythene bag in which the collected sample was kept. Afterward, the sample was washed in sterile seawater before being placed in the refrigerator for a while. Following this, the sample was gradually flushed with regular tap water and finally with sterile distilled water. Immediately after the sample was taken, the algal sample was quickly frozen and stored at -20°C. The seaweed was then used for further examinations after it was processed.

2.2. Extraction of secondary metabolites from *P. hornemannii*

A volume of 20 grams of powdered seaweed was weighed and subjected to 200 ml of methanol and chloroform (1:10, weight to volume), respectively. To extract the active ingredients of *P. hornemannii*, the content of the sample material was macerated for a week before the filtered extract from the sample material was condensed using the Soxhlet apparatus to extract the compounds and measure the yield of the crude extract.

2.3. Determination of yield

The yield of the chloroform and methanol extract obtained from *P. hornemannii* was determined by comparing with the fresh seaweed powder as per the earlier protocol (Jaswir et al. 2014). Accordingly, the

following formula was used to calculate the percentage yield:

Yield of the extract (%) = Methanol extract/Mass of seaweed powder x 100

2.4. Phytochemical analysis of *P. hornemannii*

To identify the phytochemical constituents present in the chloroform and methanol extracts of *P. hornemannii* (CEPH and MEPH) were analyzed using the procedures described earlier (Sadasivam 1996). The phytochemicals analyzed included carbohydrates, proteins, alkaloids, saponins, tannins, flavonoids, steroids and terpenoids.

2.4. Total phenol content (TPC)

A methanol extract of *P. hornemannii* (MEPH) was used to estimate its total phenolic content, although it was slightly modified from a previous protocol (Lim et al. 2007). A volume 3 ml reaction was prepared by adding 0.5 ml extract to the mixture containing 2.25 ml methanol (100%) and 0.25 ml of the Folin Ciocalteu reagent. The reaction mixture was incubated for 30 min and the results were obtained. On the other hand, 2.0 ml of Na2CO3 was added and incubated at 25°C for 120 min. The absorbance of the extract and the blank sample prepared using methanol at 765 nm was measured. It was decided to determine the TPC present in the MEPH using the formula, T = C*V/M

Where, T-TPC of seaweed extract (mg/g) in GAE, C-concentration of GAE mg/ml, and V-volume of extract (ml) and M is the weight of MEPH (g).

2.6. Total flavonoid content (TFC)

With slight modifications to the earlier methodology (Ribarova et al. 2005), the TFC was estimated from the MEPH. After adding 1 ml of seaweed extract to a volumetric flask that contained sterile-distilled water (4 ml), 0.3 ml of 5% NaNO2 was introduced and allowed the mixture to incubate for 5 minutes. Following that, 0.3 ml of 10% AlCl3 was added and incubated for 6 minutes, which was followed by 2ml of l M NaOH was used, and then distilled water was poured to make the final volume of 10ml. The UV-Vis Spectrophotometer from Shimadzu, Japan was employed for measuring the absorbance of the reaction mixture with samples and negative control at 510 nm.

2.7. FTIR analysis of *P. hornemannii*

An infrared spectrometer (IR) is used to identify the different phenolic compounds. The methanol extract of the seaweed, *P. hornemannii* was considered for functional group predictions by the standard gallic acid. The analysis was done using the SHIMADZU-FT-IR instrument (Vijayabaskar and Shiyamala 2012).

2.8. GCMS analysis of *P. hornemannii*

GCMS analysis of the compounds from *P. hornemannii* was conducted using an Agilent GC-MC-5977 MSD with a triple-axis detector equipped with an autosampler. Mass spectrometer was operated in the electron impact mode at 70 eV with 20–500 a.m.u. scan range, split ratio 5.1, and sample volume 1L. The injector temperature was set to 250°C, and the oven temperature was kept at 75°C for 3 min, before rising

at a rate of 14°C/min-1 to 250°C. A mass spectrum can be identified using the NIST library (NIST 11 - Mass Spectral Library, 2011 version) as long as it meets 80% of the critical factor (Musharraf et al. 2012).

2.9 Antioxidant activity of *P. hornemannii*

2.9.1 ABTS inhibition assay

Using a standard procedure previously designed by Re et al. (1999), we have tested the ability of the extract to scavenge radicals produced by ABTS. This study was performed on ABTS prepared by mixing 5 mL of 7 mM ABTS with 88 µl of 140 mM $K_2S_2O_8$. It was stored at room temperature in the dark for 16 h. At 734 nm, the absorbance of the samples was measured after diluting it with 50% ethanol. The radical scavenging activity of ABTS was determined by adding 0.1 ml extract (31.25, 62.50, 125, 250, and 500 µg) to 5 ml ABTS solution using an absorbance of 0.7 ± 0.01, respectively. After that, the final absorbance was measured by a spectrophotometer at 743 nm. The radical scavenging activity (%) was calculated as follows: Scavenging activity (%) = $((K_0-K_1)/K_0)$ x 100, Where, K_o-Absorbance of control; K_1-Absorbance of sample.

2.9.2 Hydroxyl radical scavenging assay

The hydroxyl radicals scavenging activity of MEPH was measured using the Fe3+-Ascorbate EDTA H2O2 system to generate hydroxyl radicals (Kunchandy and Rao 1990). TBARS is formed by the hydroxyl radicals attacking deoxyribose. The reaction mixture with final volume of 1.0 mL contained 100 µl of 2-deoxy-2-ribose (28 mM K3PO4-KOH buffer, pH 7.4) 500 µl solutions of various concentrations of MEPH (31.25, 62.50, 125, 250, and 500 µg) and standard (20 mM KH2PO4-KOH buffer, pH 7.4), 200 µl (1.04 mM EDTA) and 200 µl (200 µM FeCl3), 100 µl (10 mM H2O2). Similarly, 100 µl ascorbic acid (1.0 mM) was added in place of MEPH and all the samples were incubated at 37°C for 1 h, respectively.

Radical Scavenging activity (%) = $((K_0-K_1)/K_0)$ x 100, Where, K_o-Absorbance of control; K_1-Absorbance of sample.

Where K_o - Absorbance of control; K_1 - Absorbance of the sample.

2.10 Larvicidal activity of *P. hornemannii*

2.10.1 Collection and rearing of mosquito larvae

Aedes aegypti, *Anopheles stephensi* and *Culex quinquefasciatus* mosquito larvae were collected from ICMR-VCRC Madurai, India and then they were bred in the laboratory under 14:10 h photoperiod at 27°C with 70 ± 5% relative humidity and in the meantime, the proper feed was provided.

2.10.2 Larvicidal bioassay

In terms of larvicidal bio-efficacy, the extract was evaluated in line with the standard method set forth by (WHO 2005), with minor modifications (Thandapani et al. 2018). Twenty larvae of the 4th instar were

placed in a partitioned tray which contained 200 ml of water in each compartment. In the respective portions, the MEPH was loaded in various concentrations (5, 10, 15, 20 and 25 mg/l). The mortality of larvae was evaluated during the intervals of 12, 24 and 48 hours. The mortality percentage of larvae was calculated after three replicates of experiments (Abbott 1925).

2.11 Statistical analysis

The statistical test ANOVA was performed, where the data was expressed as mean ± standard deviation (SD) of triplicates of each assay. The statistical analysis was conducted using Origin Pro version 8.0.

3. Results And Discussion

3.1 Phytochemical test of *P. hornemannii*

In the study, it was found that there are a variety of phytochemicals present in the methanol extract of *P. hornemannii*. These include protein, carbohydrate, alkaloids, phenol, saponin, flavonoids, steroids, and tannin and terpenoids are absent. In chloroform extract of *P. hornemannii*, the protein, carbohydrates, phenol, flavonoids, and terpenoids have been reported to be present while the alkaloids, saponin, tannin, flavonoids, and steroids are absent. The MEPH showed the most promising results in comparison with the chloroform extract (Table 1). Finally, the MEPH is used for further tests.

Table 1
Phytochemical analysis of *P. hornemannii*

Phytochemical test	Methanol extract	Chloroform
Protein	+	+
Carbohydrate	+	+
Alkaloids	+	-
Phenol	+	+
Saponin	+	-
Tannin	-	-
Flavonoids	+	-
Steroids	+	-
Terpenoids	-	+

3.2. Yield, TPC and TFC

An overall yield of 8.6% was found for the MEPH. The TPC of the methanol extract of the *P. hornemannii* was 1.06 ± 0.13 mg.GAE/g, as well as the total flavonoids were 1.303 ± 0.3 mg.GAE/g. Flavonoids were known to contain more bioactive compounds than phenols in the MEPH. The methanol extracts of red seaweed *Gracilaria edulis Euchema kappaphycus* and *Acanthophora spicifera*, yielded, respectively, 2.85, 3.98 and 5.01% of yields, according to Ganesan et al. (2008). In addition, similar results were observed in the methanol extracts from several brown seaweed species, as well, such as *Sargassum marginatum* (5.45%), *Turbinaria conoides* (5.76%) and *Padina tetrastomatica* (12.31%) (Chandini et al. 2008).

During the study conducted by De Quiros et al. (2010), there was a measurement of the TPC found in the CH3OH and CHCl3 extracts of *L. snyderiae* (3.6 ± 0.12 and 3.2 ± 0.41 µg.GAE/mg), respectively. A study carried out by Souza et al. (2011) found that the highest average concentration of TPC was found in an ethanol extract of *G. birdiae* (1.13 mg.GAE/g). It was previously reported that the TPC of *Fucus spiralis* could be as high as 1.15 ± 0.06 mmol GAE/g dry weight, which is equivalent to 195.6 ± mg.GAE/g extract (Peinado et al. 2014). In light of the previous studies and evidence, it appears that the natural phenolic grouped compounds in flavonoids show anticancer, anti-inflammatory, antihypertensive, antioxidative, and antiproliferative properties (Sangeetha et al. 2016) (Mahendran et al. 2021) (Maheswari et al. 2021). Furthermore, flavonoids are also capable of protecting people from the negative side effects of coronary heart disease (Xiao et al. 2011). Despite this, it has been reported that the amount of flavonoids in *Cystoseira barbara* ranges between 5 mg and 8 mg GAE/g extract (Haddar et al. 2012). Further, there have been reports of flavonoids being potent antioxidants, inhibitors of lipid peroxidation, and antioxidants against a wide range of reactive oxygen species. Therefore, flavonoids have been investigated as impending therapeutic proxies against any number of diseases. Various evidence has been presented to suggest that phytoconstituents occur in a variety of foods, like flavonoids, tannins, and polyphenols, and that these ingredients help to mitigate many diseases due to their free radical scavenging properties (Duan et al. 2006).

3.3 FTIR spectrum of *P. hornemannii*

The functional group predictions were carried out based on IR chart interpretation, the spectrum of *P. hornemannii* was observed to have ten distinct peaks. There are various functional groups present in *P. hornemannii*, such as Alkyl halides with C-Br stretch and alcohol with C-O stretch, Ether with C = O stretch, Alkane with C-H bending and C = C stretch, alkyne with C-C stretch, Alkene with C-H stretch and Amine with N-H stretches (Fig. 1). There were similar results reported from the FT-IR spectrum of *Turbinaria ornata* with the peaks ranging from 1026.16, 3414.12, 1028.09, and 3394.83 cm^{-1} respectively (Vijayabaskar and Shiyamala 2012). According to the spectra of *T. ornata*, the absorption peaks around 3300–3500 cm^{-1} for hydroxyl groups and 2850–2960 cm^{-1} for aromatic rings were characterized by phenolic compounds. In contrast, the present results demonstrated a higher amount of flavonoids.

3.4 GC-MS analysis of MEPH

The results of GC-MS analysis of MEPH revealed 20 compounds at a running time of 4.15 mins. The chromatogram of each signal was compared with the NIST library to confirm the presence of 20 different

bioactive compounds. The MEPH showed 7 major compounds. Their respective peak area (%) and bio-potentials include n-hexadecanoic acid comprised 41.88% and possess antioxidant, nematicide, pesticide, lubricant, antipsychotic and antiandrogenic (Ragunathan et al., 2019). The second major compound, 9, 12-octadecadienoic acid (Z, Z) occupies 19.49% of antimicrobial activity (Abubakar and Majinda 2016). Followed by Hexadecanoic acid, 2-hydroxy-1- (hydroxymethyl)ethyl ester (4.51%), 9-Octadecenoic acid (Z), 2-hydroxy-1- (hydroxymethyl)ethyl (4.28%), 9-Octadecenoic acid (Z)- 2-hydroxy-1-(hydroxymethyl) ethyl (3.62%), Oleic Acid (3.47%) and Hexadecanoic acid, 1- (hydroxymethyl)-1,2-ethanediol ester (3.13%) were observed as well as the minor versatile common compounds are presented in *P. hornemannii* and they are playing in many antibacterial activities. The results are shown in Fig. 2 and Table 2. Manilal et al. (2010) have supported some similar compounds observed from the red algae *Asparagopsis taxiformis* which include 9-octadecanoic acid, methyl ester; octadecanoic acid and octadec-9-enoic acid, 2,3-dihydroxy-propyl ester might possess various bioactivity. In support of the present findings another study by Deepak et al. (2019) reported 7 compounds from *Halymenia palmata* which include 2-Propanamine, Phytol, Hexadecanoic acid methyl ester, Ether, (2-ethyl-1-cyclodecen-1-yl) methyl methyl, 5-Isopropyl-6-methylhepta- 3, 5-dien-2-ol, Citronellol epoxide (Ror S) and 3-Tetradecene. Thus, the earlier reports directed some possible biological potential lies in the present seaweed *P. hornemannii*. Presently, with these hints, some biological properties have been evaluated.

Table 2
GC-MS analysis in methanol extract of *P.hornemannii*

S.No	Compound Name	R.time	Formula	Molecular weight	Area %
1	1-Cyclohexene-1- carboxaldehyde, 4-(1-methylethenyl)-	7.782	$C_{10}H_{14}O$	150.21	0.65
2	Cyclohexanone, 2-(2- nitro-2-propenyl)-	8.463	$C_{15}H_{17}NO_3$	259.30	1.64
3	Cyclohexanebutanal, 2- methyl-3-oxo-, cis-	10.276	$C_{11}H_{18}O_2$	182.25	1.79
4	Oleic Acid	10.664	$C_{18}H_{34}O_2$	282.46	2.13
5	2-Isopropyl-5-methyl-6-oxabicyclo[3.1.0]hexane- 1-carboxalde	10.989	$C_{10}H_{16}O_2$	168.23	0.78
6	Z-(13,14-Epoxy)tetradec- 11-en-1-ol acetate	11.989	$C_{16}H_{28}O_3$	268.39	1.32
7	Bicyclo[2.2.1]heptan-2-ol, 2-allyl-1,7,7-trimethyl-	14.539	$C_{10}H_{18}O$	154.24	0.84
8	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	16.990	$C_{11}H_{16}O_2$	180.24	1.49
9	Tetradecane, 2,6,10- trimethyl-	20.634	$C_{17}H_{36}$	240.46	2.31
10	Tetradecanoic acid	22.203	$C_{14}H_{28}O_2$	228.37	2.30
11	2-Pentadecanone, 6,10,14-trimethyl-	24.160	$C_{18}H_{36}O$	268.47	1.90
12	Pentadecanoic acid	24.860	$C_{15}H_{30}O_2$	242.39	2.03
13	n-Hexadecanoic acid	27.385	$C_{16}H_{32}O_2$	256.42	41.88
14	9,12-Octadecadienoic acid (Z,Z)-	29.879	$C_{18}H_{32}O_2$	280.44	19.49
15	Oleic Acid	30.105	$C_{18}H_{34}O_2$	282.5	3.47
16	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl	32.768	$C_{21}H_{38}O_4$	354.52	3.62
17	Hexadecanoic acid, 2- hydroxy-1-(hydroxymethyl)ethyl ester	33.530	$C_{19}H_{38}O_4$	330.50	4.51
18	Hexadecanoic acid, 1- (hydroxymethyl)-1,2-ethanediyl ester	34.124	$C_{19}H_{38}O_4$	330.50	3.13
19	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl	35.562	$C_{21}H_{40}O_4$	356.5	4.28
20	Trilinolein	38.475	$C_{57}H_{98}O_6$	879.38	0.45

3.5 Antioxidant activity of *P. hornemannii*

3.5.1 ABTS inhibition assay

Antioxidants are electron donors that are soluble in both hydrophilic and lipophilic media. A major advantage of the ABTS tests over the DPPH assay is that they demonstrate steric hindrance in antioxidant molecules (Demirkiran et al. 2013). Compared to the standard, P. *hornemannii* showed greater inhibition activity at maximum concentrations, e.g., results of 52.27 ± 0.3 above 6% (Fig. 3A). This is quite impressive, considering previously reported 56.84 ± 0.41% scavenging activity from *Hypnea valentiae*. Chakraborty et al. (2015) found profoundly higher ABTS scavenging activity (63.3%) from the ethyl acetate fraction of *H. musciform*, when compared to *H. valentiae* (27.9%) and *J. rubens* (11.0%). Further, some studies have shown that *G. edulis* had significantly higher antioxidant activities (40.24%) than *G. corticata* (32.65%) (Arulkumar et al. 2018). Accordingly, the present researchers have drawn the conclusion that the scavenging activity of red seaweeds could be explained by the presence of carotenes and other pigments that contain long hydrocarbon chains and aromatic compounds (Chew et al. 2008).

3.5.2 Hydroxyl radical scavenging assay

The hydroxyl radical is thought to initiate lipid peroxidation by blocking hydrogen atoms from unsaturated fatty acids (Kappus 1991). Compared to standard ascorbic acid, *P. hornemannii* showed significant activity at its maximum concentration (75.94 ± 0.3%) in scavenging hydroxyl radicals. A comparison of the hydroxyl radical scavenging activity of the algal extract and standard is shown in Fig. 3B. Researchers found that *Kappaphycus alvarezii* and *Kappaphycus striatum* have significant antioxidant properties when dissolved in various solvents and fractionations (Diyana et al. 2015). Additionally, several other red seaweeds, which have scavenging potentials, were also identified in this study, including *Hypnea musciformis* (43.01 ± 0.81 mg/mL), *Hypnea valentiae* (32.75 ± 1.03 mg/mL), and *Jania rubens* (27.63 ± 1.36 mg/mL) (Fayaz et al. 2005) (Aboul-Enein et al. 2003)

3.6 Larvicidal activity of MEPH

The larvicidal potential of MEPH against three mosquito species, including *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* was confirmed by the lethality of larvae produced by the secondary metabolites. From the various concentrations of MEPH, we observed considerable mosquito larval mortality that lasted for up to 24 h. The larval mortality (%) produced by the CEPH and MEPH were presented in Fig. 4. Overall the MEPH showed maximum mortality (100%) in *Cx. quinquefasciatus* at the maximum concentration (25ppm) followed by An. stephensi (71%) and Ae. Aegypti (44%). Contrastingly, no significant mortality was recorded from the mosquitoes treated with CEPH. Notably, CEPH produced 58% of *Cx. quinquefasciatus* and 35% of *An. stephensi* larval mortality. MEPH produced lethality in terms of the LC50 value of 33.100 (23.083–82.757) and the LC90 value of 310.447 (108.917-6627.187) of *Aedes*

aegypti. In the following experiment, *Anopheles stephensi* was found to be susceptible with an LC50 value of 9.805 (6.196–12.971) and LC90 value of 117.075 (54.406-1060.957). Moreover, the maximum lethality value of *Culex quinquefasciatus* was also determined based on the methanol extract, with LC50 values of 2.476 (0.769–4.019) and LC90 values of 13.207 (10.1212–18.170) ppm. As can be seen in Table 3, the results are summarized. There is some evidence that a methanol extract of *P. hornemannii* exhibited some larvicidal activity against *Cx. quinquefasciatus* indicating a specific potential.

Table 3
Larvicidal activity of *P. hornemannii*

Sample	Larva	LC_{50} (mg/l) (LCL-UCL)	LC_{90} (mg/l) (LCL-UCL)	χ^2	*df*
CEPH	*Ae. aegypti*	116.194 (48.428-10010.391)	1404.277 (218.032–2.524)	2.410	13
	An. stephensi	68.596 (34.499-1559.943)	1215.955 (202.551-704.204)	1.702	13
	Cx. Quinquefasciatus	21.264 (15.415–44.853)	335.975 (102.709-21384-153)	2.654	13
MEPH	*Ae. aegypti*	33.100 (23.083–82.757)	310.447 (108.917-6627.187)	1.195	13
	An. stephensi	9.805 (6.196–12.971)	117.075 (54.406-1060.957)	1.313	13
	Cx. Quinquefasciatus	2.476 (0.769–4.019)	13.207 (10.272–19.127)	10.139	13

LC_{50}-Lethal concentration kills 50% of the exposed larvae; LC_{90}-Lethal concentration kills 90% of the exposed larvae. LCL-Lower confidence limit, UCL- Upper confidence limit, χ^2 Chi-square value, *df* = degrees of freedom.

Halymenia palmata and its fractions have been studied previously for their larvicidal capabilities. Research has shown that the plant's wet root extract, when used in conjunction with its flavonoids, is very effective against the larvae of the fourth instar of *Aedes aegypti*, followed by the larvae of the third, second, and the first instars. Patil et al. (2012) found that mosquito larvae in the fourth instar have a better immune system than larvae in younger instars. During a previous study, it was shown that extracts from marine seaweed species such as *Caulerpa scapelliformis, Dictyota dichotoma, Enteromorpha clathrata, E.intestinalis*, and *Ulva lactuca* exhibited prominent larvicidal activity against *Aedes aegypti* larvae in the fourth instar with IC_{50} values of 53.70, 61.65, 85.11, 67.70, and 91.20, respectively. The LC50 and the LC90 of the extract of *C. scalpelliformis* used in a study to test the mosquito protection properties of the seaweed against larvae of two and three instars of *Culex pipiens* were determined to be 338.91 and 1891.31 (µg/mL) respectively (Cetin et al. 2010). As a result of numerous studies, it is beneficial to add hydro-ethanolic extracts of marine microalgae (*Acanthopora spicifera, D. dichotoma* and *E. intestinalis*) to

mosquito repellents in the control of mosquito-borne diseases (Beula et al. 2011). Yu et al. (2015) investigated the larvicidal properties of a sesquiterpene isolated from 15 seaweeds from northeastern Brazil against the dengue-causing mosquito, *A. aegypti*. In addition, *Laurencia dendroidea* was found to contain larvicidal properties directed at the dengue-causing mosquito. There has been plenty of research evidenced on natural insecticides from marine sources, in particular seaweeds which play an important role in biological properties due to their active components.

4. Conclusion

The marine macroalgae are well known to contain a rich set of structurally novel and biologically active metabolites. There is a possibility that some of the secondary metabolites produced by macroalgae may have bioactive properties which are of interest. *Porteria hornemannii* can benefit the organism by employing both bioactive compounds and the content of phenols and flavonoids in its methanol extract. GC-MS analysis has revealed 7 major compounds and 13 versatile compounds, which has led to significant improvement of antioxidant activity, which in turn has led to improved medical applications. There has been evidence that methanol extracts of *P. hornemannii* may possess larvicidal effects against *Cx. quinquefasciatus* in experiments. The present study provides first-hand knowledge of *P. hornemannii* intending to develop a natural source for food supplements, industrial applications, and medical compounds.

Abbreviations

Methanol (CH3OH); Chloroform (CHCl3); Methanol extract of *Porteria hornemannii* (MEPH); World Health Organization (WHO); Sodium carbonate (Na2CO3); Potassium dihydrogen phosphate (KH2PO4); Potassium Phosphate (K3PO4); potassium hydroxide (KOH); Gallic acid equivalents (GAE); Sodium hydroxide (NaOH); Sodium nitrite (NaNO2); Aluminum chloride (AlCl3); Total phenol content (TPC); Total flavonoid content (TFC); Ferric Chloride (FeCl3); Thiobarbituric acid reactive substance (TBARS); Hydrogen peroxide (H2O2); 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS); Potassium per sulfate ($K_2S_2O_8$); Ethylenediaminetetraacetic acid (EDTA); Gas chromatography-mass spectrometry (GCMS); Fourier transform infrared spectroscopy (FTIR); Analysis of Variance (ANOVA)

Declarations

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Conflicts of Interest

The authors and corresponding authors who contributed in this research entities declares no conflict of interest for the submission in Journal of Applied Phycology.

Author's contribution

S.K. and M.A. planned and executed the framed objectives, S.K. wrote the main manuscript, B.G. made the manuscript to meet journal standards and advised critical points in the manuscript, V.M. helped out to perform the GC-MS and A.L. and N.P. helped in sample collection and performing some supportive experiments.

Data availability Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

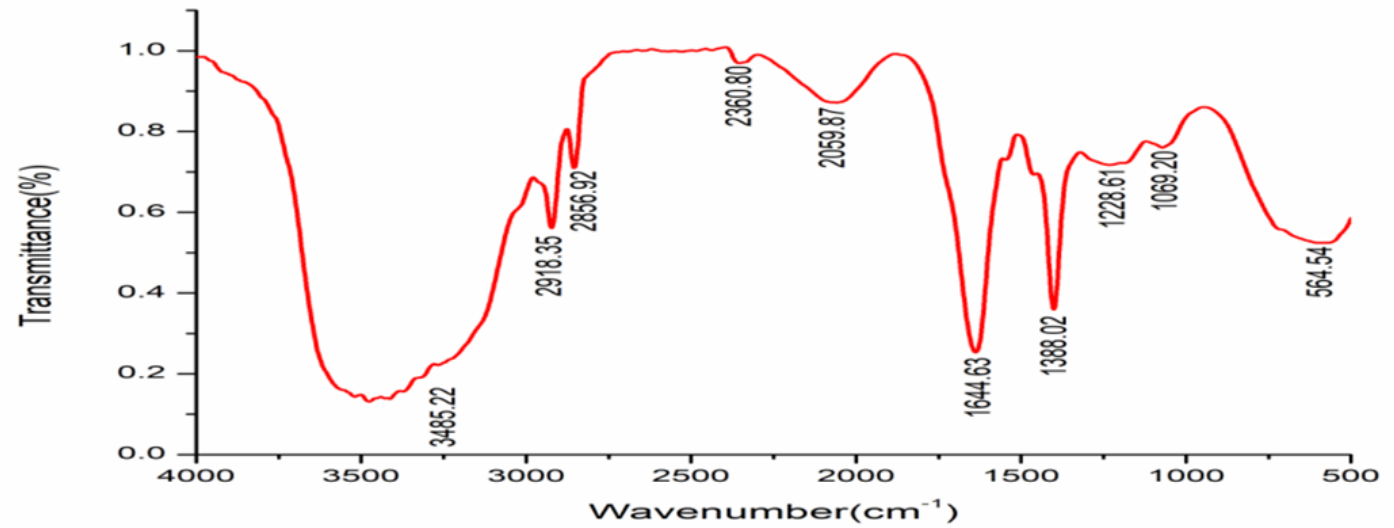

Figure 1

FT-IR analysis in Methanol extract of *P. hornemannii*

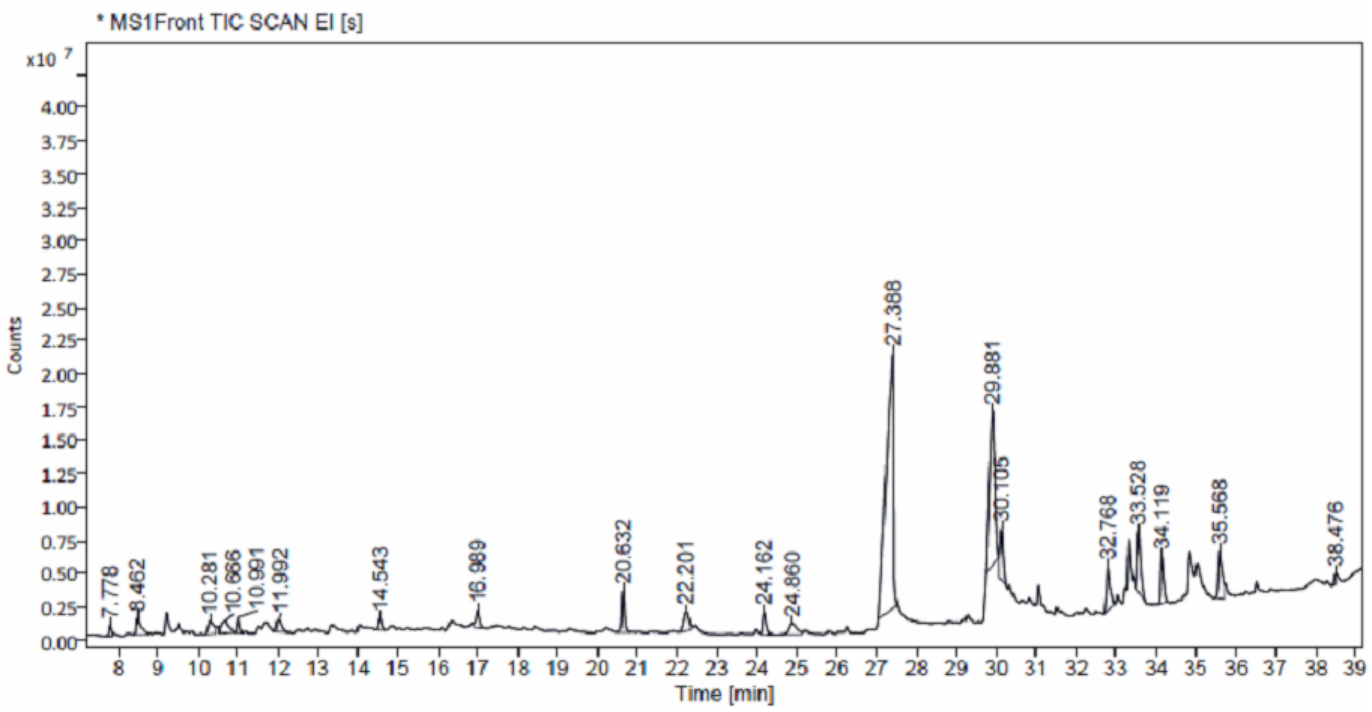

Figure 2

GC-MS analysis in Methanol extract of *P. hornemannii*

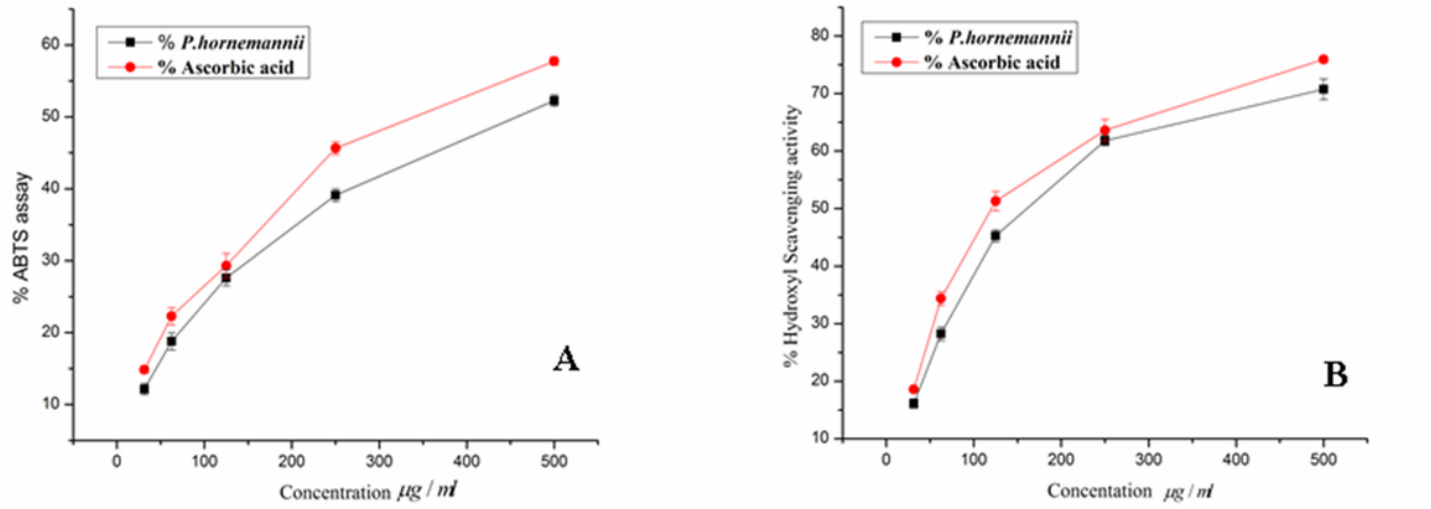

Figure 3

Antioxidant assay **A.** ABTS inhibition assay and **B.** Hydroxyl radical scavenging assay

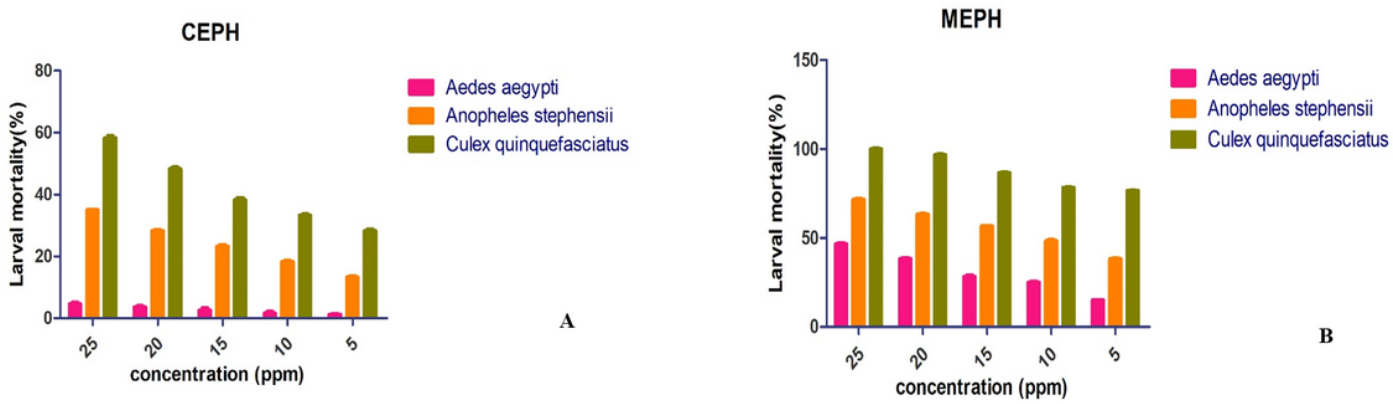

Figure 4

Percentage of larval mortality produced by the extracts: **A.** Chloroform extract of *P. hornemannii* (CEPH); **B.** Methanol extract of *P. hornemannii* (MEPH)